1970

A Pseudomonas Profile of a General Hospital's Intensive Care Unit /

Daniel David Drollette
University of Massachusetts Amherst

A *PSEUDOMONAS* PROFILE OF A GENERAL HOSPITAL'S INTENSIVE CARE UNIT

By

Daniel D. Drollette

B. S., Cornell University 1961

Thesis Submitted to the Graduate Faculty
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Department of Plant and Soil Science
University of Massachusetts
Amherst, Massachusetts

June 1970

A PSEUDOMONAS PROFILE OF A GENERAL HOSPITAL'S INTENSIVE CARE UNIT

A Thesis

By

Daniel D. Drollette

Approved as to style and content by:

(Chairman of Committee)

(Member)

(Member)

May 5, 1970
(Month) (Year)

ACKNOWLEDGEMENT

The author wishes to express sincere thanks to Dr. Warren Litsky, chairman of his thesis committee, for his encouragement and guidance. Thanks is also extended to Dr. Howard E. Bigelow and Dr. Haim B. Gunner members of the committee for their assistance, cooperation and constructive criticism in the preparation of this report.

Grateful acknowledgement is also made to Bertha Yanis Litsky for her professional advice in the development of this thesis problem.

The author is endebted to the Holyoke Hospital for the use of their facilities, financial support and compensatory time necessary to carry out this research.

Finally, public recognition is given to the author's wife, Barbara, for her loving faith and interest in this endeavor.

TABLE OF CONTENTS

I N T R O D U C T I O N

During the past ten years increasing attention has been given to the presence of Pseudomonas in the hospital environment. Throughout the antibiotic era of the nineteen fifties, this group of gram-negative organisms received little consideration as attention was mainly focused on the gram-positive cocci. The ensuing development of synthetic antibiotics tended to control these latter organisms and alleviate the seriousness of infections caused by gram-positive bacteria. It was only then that attention was focused on the gram-negative bacilli as possible agents of nosocomial infection. A frequent offender was Pseudomonas. Though this organism is not as virulent as the gram-positive cocci, it is notoriously resistant to antibiotic treatment and the toxicity of the drugs which are effective presents a therapeutic dilemma to the physician.

This study was initiated as the result of a series of Pseudomonas cross infection episodes in the intensive care unit of a general hospital. The intent of this study was to elucidate the colonizing characteristics of this organism and the longevity of this colonization. Previous studies have focused on a particular object or piece of equipment that was subjected to many variables or conditions throughout the hospital environment. The design of this investigation was to comprehensively study a relatively small,

contained unit within the hospital complex and to obtain as much data as possible in terms of its total environment.

REVIEW OF THE LITERATURE

For some time after its discovery, Pseudomonas was regarded as a harmless saprophyte. In 1882 this organism was implicated for the first time as a cause of human infection by Gessard in his now "classic" study of infected wounds draining "blue green pus"(29). Since that time the blue pus phenomenon has been widely observed and its agent, Pseudomonas aeruginosa established as the cause. However, from that beginning to the present, clinical literature has relegated little more than a fleeting paragraph to this genus and there has been a reluctance to label any Pseudomonas species as an outright pathogen. Moreover there has never been any agreement as to the number of Pseudomonas species which may be agents of human infection(7,26,29).

In 1933 Park and Williams(22) indicated that Ps. aeruginosa is usually a commensal organism which assumes a degree of pathogenicity toward severely debilitated patients. Jordan and Burrows(10) were more definitive by stating: "Apart from the many doubtful cases in which Ps. aeruginosa is found mixed with streptococci, staphylococci, and other microorganisms where its share in inciting pathological processes is problematic, numerous instances are on record in which little or no question exists as to its etiologic role."

In 1949 Frobisher(7) stated that this organism probably does not have the power of invading healthy tissue but seats itself in already diseased foci continuing to exist as a saprophyte. Later, Zinsser(26) suggested its pathogenicity is determined more by the resistance of the host than by its inherent virulence. Topley and Wilson(29) label the species a pathogen of low virulence but an important cause of hospital acquired infection.

Two central facts regarding Pseudomonas emerge from this review: (i) Ps. aeruginosa and other species are now considered pathogens capable of initiating the infection process but possessing little inherent virulence and (ii) these organisms are host selective and the most susceptible group are the severely debilitated patients.

As these facts became established, hospital acquired infections continued to receive increased attention. The gram-positive cocci, especially the penicillin resistant staphylococci, received the most attention in the nineteen fifties. Toward the end of that decade the drug industry responded by introducing several synthetic penicillins; this coupled with warnings against the indiscriminate use of antibiotics apparently quelled the crises of the gram-positive cocci.

During the early nineteen sixties, the pattern of hospital acquired infections appeared to be changing in that the gram-negative bacilli were becoming increasingly prominent and practically all of the enteric bacilli were implicated in various episodes. Fortunately, however, the enterics have continued to remain relatively sensitive to gram-negative specific and broad spectrum antibiotics. Pseudomonas, on the other hand, has continued to remain essentially resistant to most of these drugs. Only two Pseudomonas specifics, colistin and polymyxin B, have remained effective in spite of toxic effects which dictate their restricted use(23).

It was not surprising, therefore, that increased attention was given to the Pseudomonas problem by hospital sanitarians and clinicians. Starting in the nineteen sixties, the Pseudomonas issue prompted a number of investigators to study the occurence and etiologic significance of this organism in the hospital environment.

In 1961 Sandusky(23) reported on a study concerned with the occurence of post-operative Pseudomonas infection. During this five year investigation he observed that most infections implicating this organism were a complication of surgical and related therapeutic procedures. Though he did not find the relative incidence to be increasing, he did conclude that the hospital environment was a reservoir

abundant with Pseudomonas. Following this and similar reports, a number of studies were initiated to reveal the specific habitats in this reservoir and the transmission routes between these habitats and the susceptible patient.

The habitats revealed were sometimes startling. Lee and Fialbow(13) reported the well known benzalkonium chloride disinfectant to be a source of Pseudomonas. Air conditioning systems were responsible for the dissemination of Pseudomonas in some hospitals as reported by Anderson(1). Moffet and Williams(18) published an article on the recovery of bacteria from distilled water and found the carboys contaminated on every occasion of testing. Pseudomonas was a significant finding from all samplings. Tap water and deionized water, on the other hand, were sometimes sterile. Along with this, one must consider the report by Cross, Benichimol, and Dimond(3) who demonstrated that the faucet aerator can be a significant source of Pseudomonas.

Urine bottles and bedpans have always been held as vehicles of microbial transmission by hospital epidemiologists and McLeod(15) demonstrated that these objects were potential reservoirs of Ps. aeruginosa. This finding was further buttressed by Shooter, Walker, Williams, Parker, Ashechov, and Bullimore(25) who found 38% of 249 patients studied harbored Pseudomonas in their feces. Data was also presented which indicated a correlation between fecal

carriage and a history of intensive antibiotic therapy or colon surgery.

Several studies have implicated hand creams as potential sources of Pseudomonas. Morse and Schonbeck(19) isolated Pseudomonas and several other gram-negative bacilli from both used and freshly opened dispensers. Recovery was made from hospital manufactured creams as well as nationally distributed products. In a related study, Morse, Williams, Grenn, Eldridge, and Rotta(20) confirmed six cases of septicemia resulting from a hand cream dispenser. The causative organism was Klebsiella pneumonia.

Respiratory apparatus has been the subject of numerous studies relating to Pseudomonas cross infection. Corrugated rubber tubing, diaphragms, valves, nebulizers, water reservoirs, nasal cannulas, endotracheal tubing, pumps and a host of other increasingly complex components make this equipment a prime source or reservoir for this species. The problem appears to be twofold: (i) to initially administer this apparatus Pseudomonas-free, and (ii) to maintain this condition while the equipment is being used either continually or intermittently by a particular patient. To a degree, this problem has been circumvented through use of some disposable parts.

Kundsin and Walter(12) warned that inhalation therapy equipment deals with the most highly colonized portion of the body, becomes readily contaminated, and is often casually disenfected or completely neglected. They recovered a wide variety of gram-negative bacilli including Pseudomonas as well as viruses and fungi from the moist, dark interiors of the corrugated rubber tubing. Contaminated resuscitators for newborns were shown by Fierer, Taylor and Gezon(6), and Moffet, Allen and Williams(17) to harbor Pseudomonas. Mertz, Scharer and McClement(16) recovered this organism from preparations to be aerosolized through inhalation therapy equipment. Sutter's(28) study showed that a majority of the patients probed with a endotracheal suction catheter became infected with Pseudomonas and half of the patients developed significant Pseudomonas infection.

Specific units designated for burn therapy are also subjected to Pseudomonas colonization. Lowbury and Fox(14) studied a burns unit for reservoirs of Pseudomonas and reported that although inanimate objects often harbored the organism, it was the infected patient who consistently yielded the highest counts.

Hurst(9) investigated the survival of Pseudomonas in a vacated burns unit and reported a rapidly dwindling population of the organism in the absence of patients. The unit

was temporarily created for an emergency to handle 25 burned patients all of whom became heavily colonized. It was occupied for 27 days and then closed down. Bacteriological testing was done on the floor and showed counts of two to three thousand colonies of Ps. aeruginosa per ml of mop water during the patient occupation period. Eight weeks after closing of the ward the count decreased to zero. The phage type of the floor isolates were identical to those that had colonized the patients.

Epidemiological studies including those cited above generally involve some system of typing the organisms. In the case of Pseudomonas three systems are presently in use as reviewed by Osman(21). Serological methods are based on several somatic and flagellar antigens. These techniques are laborious and time consuming. Phage typing, although plagued with a considerable number of untypable strains, is still popular. Pyocine typing, based on the inhibitory effects of a small group of pyocines on the growth of Pseudomonas, is also widely employed. The pyocine method is based on the earlier developed colicine scheme of typing strains of Escherichia coli. The pyocine method is being increasingly favored in the study of the epidemiology of Pseudomonas infections, since the method is simpler than either the phage or the serological technique.

One of the prime considerations in any bacteriological study is the choice of media. In the case of Pseudomonas several selective and many general purpose media have been devised. Considering the vast array of substrates metabolized by the Pseudomonas genus, many highly selective media types are available to the worker. Cetrimide media, widely used for this work, has the limitation of inhibiting most species of Pseudomonas other than Ps. aeruginosa.

Q medium developed by Drake(5) is less inhibitory toward Pseudomonas while it inhibits most gram-positive organisms and many gram-negative bacilli. Some enterics do grow on it but these colonies develop much slower than the pseudomonads. Detection is thus somewhat facilitated. The selective substrate is ethyl alcohol. Stanier(27) demonstrated that approximately seventy per-cent of the Pseudomonas species utilized ethyl alcohol as a sole carbon source.

Non-selective media for Pseudomonas isolations include Pseudomonas F agar (Difco) and Pseudomonas P agar (Difco) which reveal the fluorescent and pigment producing pseudomonads. Together, these two media would detect most of the Pseudomonas species as indicated by King, Ward and Raney(11).

EXPERIMENTAL PROCEDURES AND RESULTS

This study was performed in four phases: (I) selective and differential media were evaluated for their recovery of Pseudomonas, (II) the pyocine technique was tested and perfected, (III) the environment was sampled during a period free of clinically diagnosed Pseudomonas infections, and (IV) a replicate of Phase III but in the presence of a fulminating Pseudomonas case.

Phase I. Medium Evaluation

Considering the variety of organisms existing in the environment and the numerous samples required for this study, a selective medium was required that would efficiently recover Pseudomonas yet inhibit most other species. A medium containing ethyl alcohol as a carbon source was selected for this purpose. To determine the extent that such a medium might inhibit some pseudomonads, sampling was replicated with a non-selective medium as a means of comparison. The non-selective Pseudomonas F agar (Difco) was evaluated for its fluorescein enhancing qualities of non-Pseudomonas species.

A. Pseudomonas F agar evaluation. Ps. aeruginosa and Ps. putida, maintained in the hospital laboratory stock

culture collection, were used as test organisms for this evaluation. A single colony of each organism was suspended in 1 ml of sterile saline and serially diluted in ten-fold concentrations from 10^{-4} to 10^{-9}. Using a calibrated pasteur pipette, 0.1 ml portions of each dilution were inoculated and streaked with glass rods on 3 plates of Tryptose Blood Agar Base (Difco) and 3 plates of Pseudomonas F agar; the former serving as a control. The plates were incubated at 37°C for 48 hours and colonies enumerated and characterized under an ultra-violet lamp.[1]

Results of Pseudomonas F agar evaluation. Pseudomonas F agar recovered somewhat fewer organisms of both species tested. At a dilution of 10^{-6}, 84 colonies of Ps. aeruginosa were isolated as compared to 109 on Tryptose Blood Agar Base. At the same dilution 69 colonies of Ps. putida were isolated from Pseudomonas F agar as compared to 103 from Tryptose Blood Agar Base. Colonies of both species produced evident and readily detectable fluorescence.

B. Q Medium evaluation. Q medium was evaluated for its fluorescent enhancing and Pseudomonas inhibiting qualities by the same methods employed for Pseudomonas F agar. The dilution range, however started at 10^{-6}.

---

1. Blak-Ray, model UVL-22, Ultra Violet Products, Inc., San Gabriel, Calif.

The inhibitory activity of this medium against non-Pseudomonas species was also evaluated. Six species of bacteria (Escherichia coli, Aerobacter aerogenes, Serratia marcesans, Bacillus subtilus, Staphylococcus aureus and Streptococcus viridans) were grown on the surface of a Tryptose Blood Agar plate. After 24 hours one colony of each species was suspended in separate 10 ml volumes of Brain Heart Infusion Broth (Difco). Each suspension was subsequently diluted to three concentrations: 10^{-4}, 10^{-6}, and 10^{-8}. One tenth ml of each dilution was delivered to sterile petri dishes to which sterile Q medium cooled to approximately 45°C was added. Mixing was affected by swirling prior to solidification. Plates were incubated for 72 hours and colonies enumerated. An unltraviolet lamp was used to detect any fluourescence produced.

Results of Q medium evaluation. Q medium gave good recovery of both species tested as shown on Table 1. Fluorescence was readily detectable but not evident as on Pseudomonas F agar. The selectivity of Q medium is shown on Table 2. Gram-positive cocci were inhibited; gram-positive bacilli were present in small numbers and spreading was largely arrested.

TABLE 1. Comparison of *Pseudomonas* recovery on Q medium and Tryptose Blood agar.

Organism	Dilution	Average Plate Count	
		Blood agar	Q medium
Ps. aeruginosa	10^{-6}	156.7	185.7
	10^{-7}	15.0	27.3
	10^{-8}	2.3	3.7
	10^{-9}	0	0.3
Ps. putida	10^{-6}	23.3	19.7
	10^{-7}	1.0	4.7
	10^{-8}	0	0
	10^{-9}	0.3	0

TABLE 2. Growth of several non-*Pseudomonal* species on Q medium.

Organism	Relative Growth
Staphylococcus aureus	-
Streptococcus viridans	-
Aerobacter aerogenes	+ + + +
Escherichia coli	+ + + +
Bacillus subtilus	+ +
Serratia marcescens	+ + + +

Phase II. Pyocine Typing of Pseudomonas

The media tested for pyocine typing included Meuller Hinton (Difco), MacConkey (Difco), Pseudomonas F (Difco), and Tryptose Blood Agars (Difco). The Meuller Hinton medium presented the most discreet zones of inhibition and was employed for further study.

The strain to be typed (a producer strain) was streaked diametrically across the Meuller Hinton medium to produce a band of inoculum approximately 1 cm wide. The plates were first incubated at 37°C for 24 hours as suggested by Darrell and Wahba(4); these conditions yielded poor results and the incubation procedure was changed to 32°C for 16 hours as advocated by Gilles and Govan(8). The growth was then removed as completely as possible by scraping with a sterile microscope slide and sterilized by placing the open inverted plates over cotton gauze soaked with chloroform for fifteen minutes. The plates were then turned over and aired for several minutes to eliminate traces of chloroform in preparation for the final streaking.

Cultures of the six indicator strains, incubated for 24 hours at 37°C in Brain Heart Infusion broth, were streaked at right angles to the original producer strain. Completed, these plates had six distinct inoculation bands and were incubated at 37°C for 24 hours. It was during

this period that the pyocine remaining from the producer strain engaged their antibiotic effect on the indicator strains (figure 1).

Results of pyocine typing. Table 3 shows the provisional typing scheme used to identify isolates from this study. If pyocine production was not detected by the six indicator strains chosen for this investigation, the isolate was grouped as untypable.

Phase III. Environmental Sampling Prior To A Pseudomonas Case

The diverse number of areas, situations, and levels of contamination existing within the intensive care unit necessitated the design of a sampling scheme geared to these conditions. As a result, the unit was divided and specific methodology was adapted to each of the following five categories:

A. Surfaces

B. Personnel

C. Water and water reservoirs

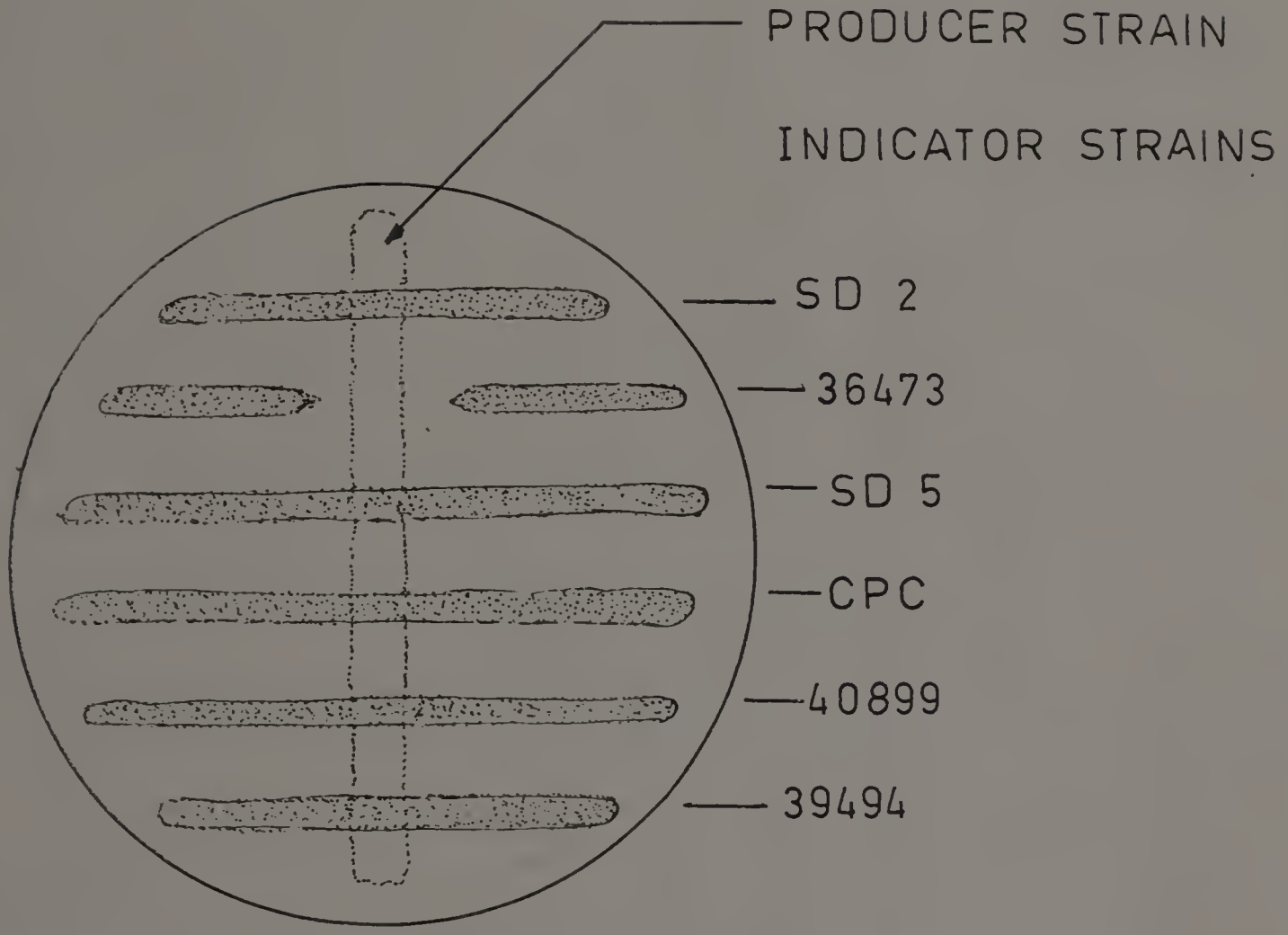
D. Solutions and medications

E. Inhalation therapy

This phase of the sampling was implemented to determine the possible reservoirs of Pseudomonas during a period when patients in the unit showed no evidence of Pseudomonas

FIGURE 1

A TYPE D PYOCINE PATTERN



NOTE Only strain 36473 is inhibited in this profile.

Table 3. Provisional types of pyocine patterns.

Type	Indicator Strains					
	SD 2	36473	SD 5	CPC	40899	39494
A	+	+	-	-	+	+
B	-	-	+	-	+	-
C	-	-	+	-	-	+
D	-	+	-	-	-	-
E	-	-	-	-	-	+
Untypeable	-	-	-	-	-	-

\+ Inhibition of the indicator strain

\- No inhibition of the indicator strain

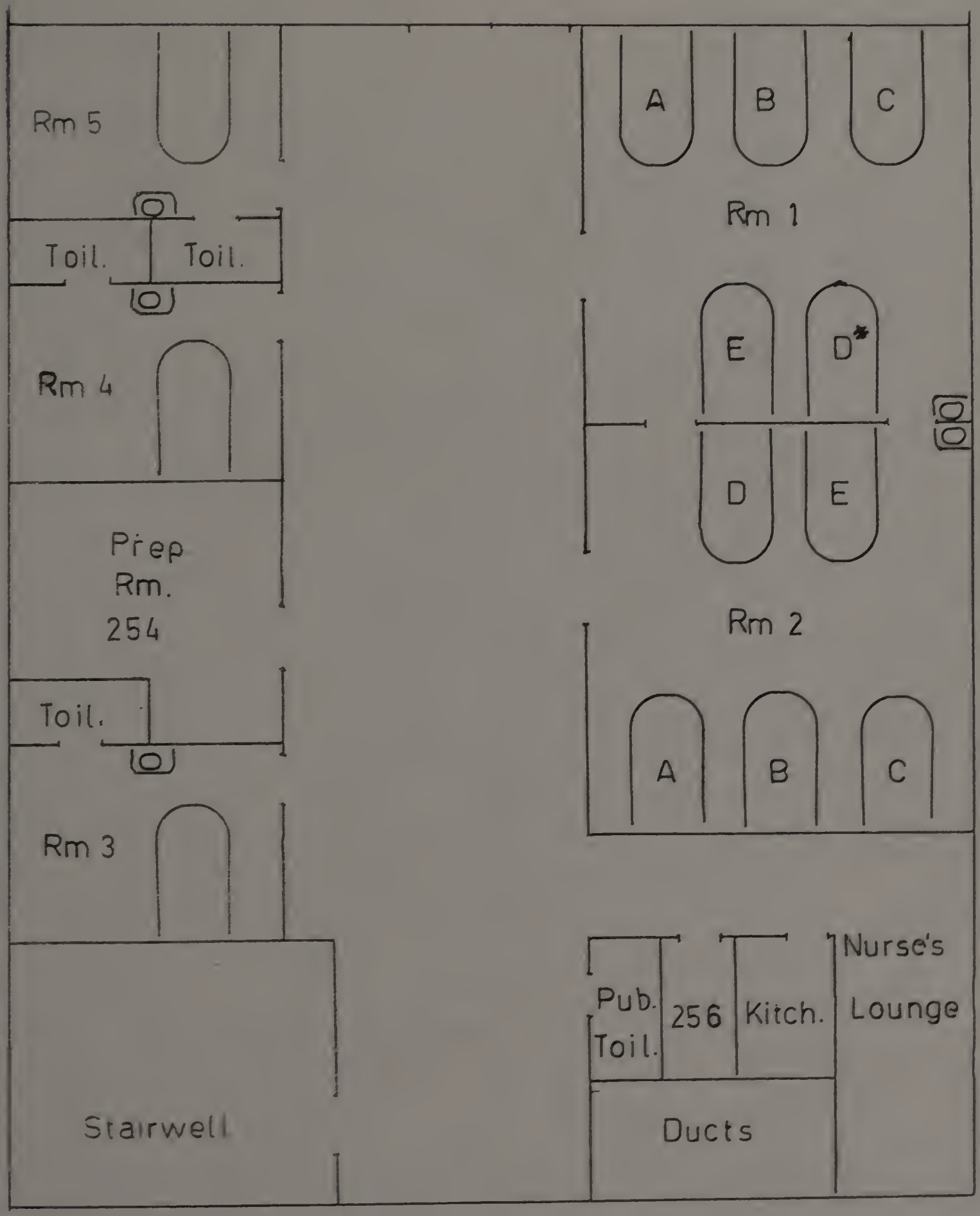
infection. The data from the above catagories comprise a "base level" which was later compared to the data obtained after a fulminating Pseudomonas case occupied the unit.

The results from each catagory are presented with notation as to the specific article tested and its room location within the unit. Instances where Pseudomonas was isolated are followed by a pyocine type letter. On the floor plan (figure 2) the two large wards, Rooms 1 and 2, which are prime areas of patient care and personnel activity should be noted. Rooms 3, 4, and 5 are critical rooms used for certain terminal cases, for isolation cases, and when wards 1 and 2 are filled to capacity. The preparation room #254 is also a focal point where bed pans are dumped, mop water is discarded, gowns and linens are collected and stock solutions of electrolytes and saline are stored. The personnel lounge, lavatory and kitchenette also receive a considerable flow of activity.

A. Method of sampling surfaces. Bed linens were sampled following a technique suggested by Davidson and Wells(24). Four standard size petri dishes (100 mm diameter), 2 filled with Q medium and 2 filled with Pseudomonas F agar were uncovered, placed with the open face toward the bed linen and vigorously swept with 10 strokes in an arc approximately one foot long. All other surfaces were sampled using the direct contact (Rodac) plate. Ten plates

Figure 2

Floor Plan - Intensive Care Unit



* Pseudomonas case located here

TABLE 4. Prepatient phase: recovery of Pseudomonas from surfaces.

Area	Total Count Q medium	Total Count Pseudomonas F Medium
Room 2 floor	6 type D	0
Room 3 floor	2 untypeable	0
Room 254 sink basin	1 untypeable	0

bag was then tied off and returned to the laboratory. Two-tenths ml of this water was inoculated over the surface of each of 5 Q medium plates and 5 Pseudomonas F agar plates. The plates were incubated for 72 hours at 37°C and counted under an ultraviolet lamp.

Results of personnel cultures. In no instance was Pseudomonas recovered from either the hands or stools of the nursing personnel studied in this preliminary testing period.

C. Method of sampling water and water reservoirs. Using a sterile pipette, a 5 ml sample of water was obtained at each of the following sources and placed in a sterile screw cap tube: Sink drains, toilet bowls, tap water, suction traps, oxygen nebulizers, and melted ice. Each sample was vigorously shaken and 0.2 ml aliquots were delivered to the surface of each of 5 plates of Q medium and 5 plates of Pseudomonas F agar. The inoculum was spread with a spreader rod and the plates incubated at 37°C for 72 hours. Colonies were enumerated under an ultraviolet lamp.

Results of sampling water and water reservoirs. Table 5 reveals the Pseudomonas potential that water reservoirs harbor. Sink drains and toilet water appear to become readily colonized with pseudomonads. Those samples which yielded Pseudomonas were usually from the more stag-

TABLE 5. Prepatient phase: recovery of Pseudomonas from water reservoirs.

Area	Total Count Q Medium	Total Count Pseudomonas F Medium
Room 3		
sink drain	0	2 type C
toilet water	1 type A	3 type A
Room 4		
sink drain	TNTC un-typeable	TNTC un-typeable
toilet water	TNTC un-typeable	TNTC un-typeable
Room 5		
sink drain	37 type D	30 type D
toilet water	TNTC type D	TNTC type D

nant reservoirs which were less frequently disinfected or flushed.

D. Sampling of solutions and medications. Considering the daily routine of dispensing these preparations, it would seem probable that they would eventually become contaminated. Hand creams evaluated by Morse et al(19) contained a wide variety of microorganisms. It would follow that other preparations similarly dispensed might also become contaminated.

To test the possibility that Pseudomonas may colonize these preparations, aliquots of approximately 2 ml were diluted 10^{-1} and 10^{-2} in sterile water to diminish the bactericidal effect. Two-tenths ml of aliquots were delivered to the surface of 5 plates of Q medium and 5 plates of Pseudomonas F agar. The inoculum was spread over the surface and the plates incubated at 37°C for 72 hours. Colonies were enumerated under an ultraviolet lamp.

Results of sampling solutions and medications. In no instance was Pseudomonas isolated from these sources. This may be due in part to the limited use of refillable containers and the increasing popularity of single-use-then-discard items. In this study only three preparations were found in refillable stock bottles: ethyl alcohol 70%, distilled water, and the liquid soap. The hand lotion used in this hospital is not manufactured on the premises; it has

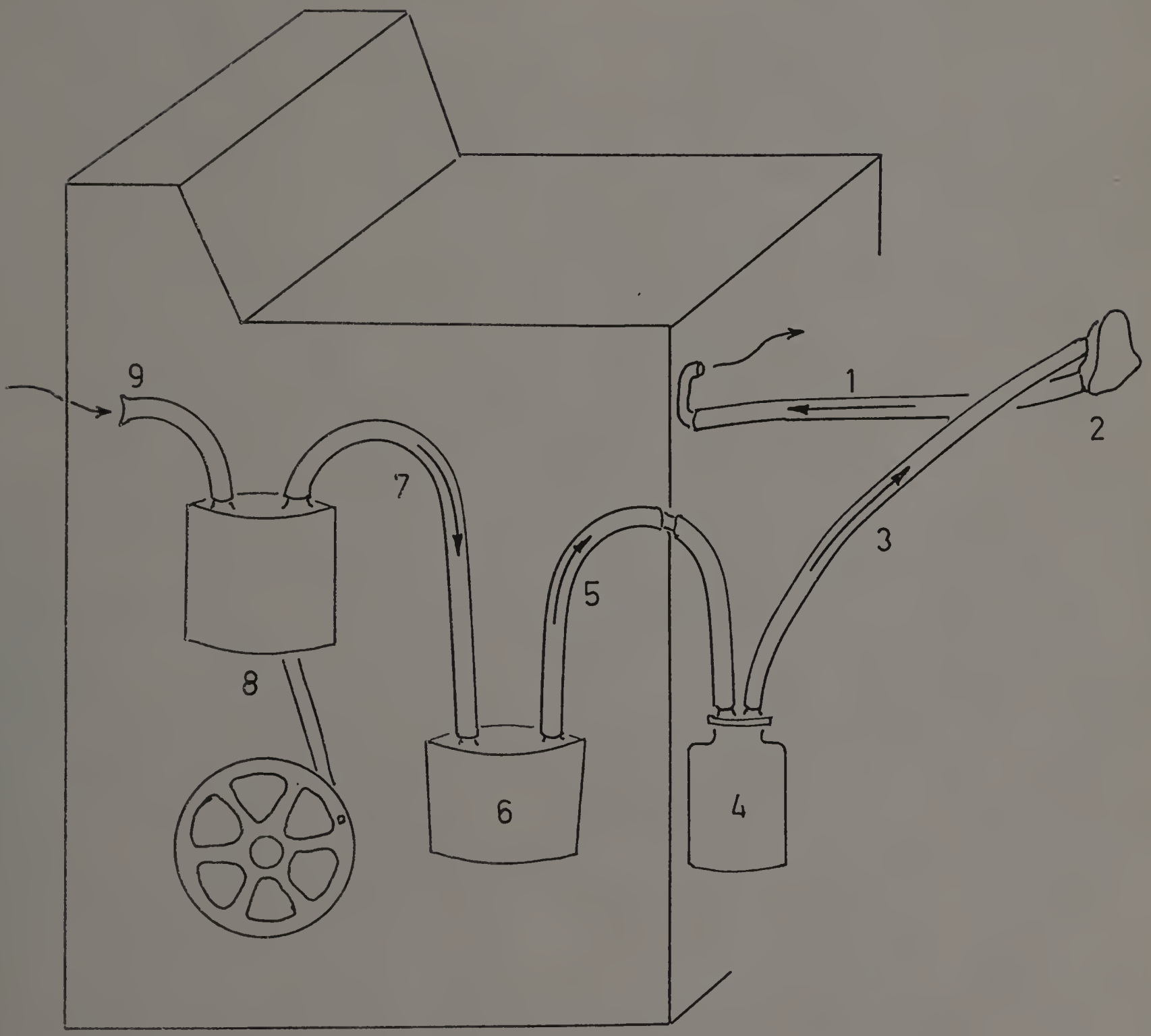
been tested on several occasions other than this study and in no instance has Pseudomonas been isolated.

E. Method of sampling inhalation therapy equipment. The sampling technique for this aspect of the study was largely improvised. Standard methods have not been formulated for the many intricate devices employed in this specialized equipment. The apparatus used most extensively in this unit and used on the terminal Pseudomonas case to be detailed in the final phase of this study was the intermittent positive pressure machine (figure 3). This device is used on severe cases unable to maintain the natural rhythm of respiration. A forced draft of room air or oxygen is intermittently administered to the patient with a face mask or through a tracheostomy tube. The volume and rate is adjustable.

The apparatus was disassembled and each element sampled employing a predetermined sequence. Those parts making contact with the patient were always sampled first and those furthest from the patient sampled last. The machine was disassembled into six elements: exhale tubing, inhale tubing, reflux water trap, tubing from trap to nebulizer kettle, nebulizer kettle and inlet pipe. From the kettle and trap 250 ml of water was drawn directly and tested. The other parts were flushed with 250 ml sterile tap water which was poured into a sterile funnel attached to

FIGURE 3

THE INTERMITTENT POSITIVE PRESSURE RESPIRATOR



LEGEND

1. Exhale tubing
2. Facepiece
3. Inhale tubing
4. Reflux trap
5. Trap-kettle tubing
6. Kettle
7. Kettle inlet
8. Pneumatic pump
9. Air intake

one end of the tubing and collected in a sterile polyethylene bag secured at the opposite end. In the laboratory these samples were filtered through a membrane filter (Millipore 0.45 mu pore size) which were aseptically transferred to 4 oz sterile specimen jars containing 10 ml sterile Brain Heart Infusion broth. These jars were then vigorously shaken 100 times. Two-tenths ml of this broth was then transferred to each of 5 plates of Q medium and 5 plates of Pseudomonas F agar. The plates were incubated at 37°C for 72 hours and colonies enumerated under an ultraviolet lamp.

Results of Inhalation Therapy Equipment Sampling.

Sampling of inhalation therapy equipment both before and after patient use revealed no Pseudomonas contamination. Gram-negative bacilli, usually Flavobacterium, were a frequent finding from the machine after patient use. Before use (shortly after disinfection) the machine yielded uniformly negative cultures.

Phase IV. Environmental Sampling During And After A Pseudomonas Case

This phase of the study commenced on May 14, 1969 approximately one month after the pre-Pseudomonas case level had been established. The patient, W. G., a 58 year old

male was admitted to the hospital on April 26, 1969 for treatment of a duodenal ulcer complicated with diabetes and neuritis. He was placed on a medical-surgical ward and remained there until surgery was performed. The surgery, accomplished on May 9, consisted of pylorroplasty and a vagotomy after which the patient began to experience respiratory distress.

That evening he was moved to the intensive care unit room 1, bed D (see Fig. 2), a tracheostomy was performed and the intermittent positive pressure machine administered. An aspiration of thick, white mucuous drawn from the lungs was sent to the laboratory for culture and sensitivity testing which subsequently yielded Diplococcus pneumoniae. On May 10, a swab from the incisional area of the tracheostomy was sent to the laboratory for culture and sensitivity testing; this yielded Ps. aeruginosa. On the same day, three blood cultures were drawn, all of which were negative after 2 weeks of incubation.

In the fourteen days that followed, the patient's health continued to deteriorate in spite of increasing medical assistance including bladder catherization, I. V. infusions, blood transfusions, hemodyalysis, peritoneal dyalysis, antibiotic administration, and respiratory therapy. Cultures sent to the laboratory during this period became increasingly positive for Ps. aeruginosa. By May 20, blood

cultures were yielding Ps. aeruginosa and the urinary tract was yielding the same organism. The patient's health continued to fail and he expired on May 24, 1969. Finally, a specimen of lung tissue aseptically excised at autopsy yielded Ps. aeruginosa.

A. Method of environmental sampling during a Pseudomonas case. The environment was bacteriologically monitored during the patient's occupance of the intensive care unit. On three separate days (May 14th, May 19th, and May 22nd) replicate samples representing each of the six catagories outlined in Plase III were obtained. The sampling was largely concentrated around the area of the patient's bed and within his ward. Occasional random samples were made at more remote areas for comparison.

Due to the limitation of time during the critical period and the desirability of replicating the sampling as often as possible, the number of plates utilized per sample was reduced from that outlined in the methodology section of Phase III. Pseudomonas F agar was omitted from this survey. Three plates of Q medium were used for testing of surfaces and 2 plates each for water, hands of personnel, and inhalation therapy equipment components.

Solutions and medications were inoculated on Q medium as outlined in Phase III omitting Pseudomonas F agar. All Pseudomonas isolates recovered from the patient and the en-

vironment were pyocine typed.

Results of sampling during a Pseudomonas case. It was demonstrated that Pseudomonas became well established in the unit (Table 6). Recovery was made with increasing frequency from a wider variety of objects tested. Water sources and inhalation therapy equipment harbored the largest numbers. The recovery rate does not appear to rise dramatically with time. The organism apparently establishes itself in any niche available soon after the source of proliferation is introduced. It remains at relatively constant levels unless unusual, favorable conditions are available.

Pyocine typing of Pseudomonas isolated directly from the patient's lung tissue, urine, and blood revealed the organism to be type E. This same pyocine type accounted for 73.5% of the total number of Pseudomonas isolations made from the environment through the overall study. Untypeable strains accounted for 10.2% of the total. Each isolate listed in Table 6 is followed by a letter designating the pyocine type of that isolation. In table 7 the instances of isolating each pyocine type are tabulated.

B. Method of sampling after a Pseudomonas case. Three replicate samples were made after the patient expired. The first post mortem sampling was performed on May 26th, approximately 40 hours after the patient's death. Sampling

Table 6

Pseudomonas recovery during and after a *Pseudomonas* case.[1]

Area	May 14	May 19	May 22	May 26	May 28	May 30
Surfaces						
Floor D rm. 1	1E	2E	43E	0	0	0
Bed table D rm. 1	0	0	8E	0	0	0
Bed spread B rm. 1	10E	9E	2E	0	0	0
Bed pan rm. 1	0	12E	3E	-	-	-
Sink basin rm. 1	0	1D	1E	5E	0	0
Sink handle rm. 1	0	0	3E	0	0	0
Personnel						
Nurse J. A.	2B	0	-	-	-	-
Nurse A. C.	-	8E	0	-	-	-
Water Sources						
Sink drain rm. 1	3E	7E	6E	TN E	TN E	TN E
Sink drain rm. 2	TN[2] E	-	-	-	-	-
Sink drain 254	TN Un	-	-	-	-	-
Sink drain 256	TN C	-	-	-	-	-
Toilet (person.) 256	2E	-	-	-	-	-

1. *Pseudomonas* was isolated from the patient's tracheostomy, blood specimens, urine specimens and lung tissue on May 10th, May 20th, May 20th, and May 24th, respectively.
2. Too numerous to count.

Table 6 (cont.)

Area	May 14	May 19	May 22	May 26	May 28	May 30
Inhalation Therapy						
Exhale tubing	TN E	TN E	TN E	0	-	-
Inhale tubing	TN E	TN E	TN E	0	-	-
Reflux trap	TN E	TN E	TN E	0	-	-
Tubing from trap to nebulizer	36E	29E	13E	0	-	-
Nebulizer kettle	16E	0	0	0	-	-
Inlet tubing	0	0	0	0	-	-

TABLE 7

Pyocine types of Ps. aeruginosa recovered from the environment

Type	Surfaces	Water Reservoirs	Inhalation Therapy	Solutions Medications	Personnel	Total
A	0	1	0	0	0	1
B	0	0	0	0	1	1
C	0	2	0	0	0	2
D	2	2	0	0	0	4
E*	12	8	14	0	2	36
Untypeable	2	3	0	0	0	5

*This was the pyocine type isolated from the patient.

was repeated on May 28th and May 30th using the same techniques as outlined for sampling during a Pseudomonas case.

Results of sampling after a Pseudomonas case. Perhaps the most startling data obtained in this study was that gained in this phase. The first sampling period, 40 hours after expiring, revealed a striking decrease in the frequency of recovery. Counts returned to zero on all objects tested except the sink drain in Room 1. Thus the unit returned to its pre-Pseudomonas case level within this short time span. Table 6 contains the data obtained during this sampling phase which extended to six days after the patient's expiration.

D I S C U S S I O N

Pseudomonas aeruginosa is a common inhabitant of the hospital and must be considered a serious problem. Cross infection in wards continues to threaten the lives of the severely debilitated. Therapy itself is attended with some risks. An intensive therapeutic regimen is accompanied by a multitude of possibilities for the acquisition of an infection as Mertz et al(16), Sandusky(23), and Sutter (28) emphasized in their studies. Patients under this kind of therapy are least able to defend themselves against secondary, complicating afflications. Where Pseudomonas is implicated there is the added possibility that the organism will be refractory to most antibiotics.

It appears from this study that Pseudomonas is largely a transient organism in the hospital. The data indicates that it colonizes water laden areas and contaminates a wide variety of objects in close proximity to sources of proliferation but disappears soon after this source is removed. This is in contrast to Hurst's study(9) where Pseudomonas survival extended 8 weeks.

In the early phase of this investigation an attempt was made to establish base levels of this organism. The data shows approximately one-half of the sink drains and toilet bowls in this unit were colonized with the organism

at that time. Pseudomonas is indeed rare on dry inanimate objects, in the absence of constant seeding. Other than the colonized water reservoirs and three instances of isolation from surfaces (floors and sink basins) the environment, as sampled, was essentially free of Pseudomonas. Furthermore, the pyocine types of these isolations were largely unrelated. This would dispute the possibility that the unit had been recently showered by some common source of contagion and leads one to suspect that Pseudomonas becomes a relatively permanent resident of stagnant water pockets.

Throughout these base level determinations the non-pseudomonal flora, as observed on Pseudomonas F. agar, consisted of a variety of organisms (some potential pathogens) and a wide range of plate counts. Fungi, especially Aspergillus, were frequently isolated from the interior of corrugated rubber tubing on respiratory apparatus. Flavobacterium was frequently isolated from the water reservoirs of the oxygen nebulizer and suction trap bottles. The personnel, in all cases, presented a well balanced normal stool flora. Hand cultures yielded essentially negative results on the media used.

The yield of Pseudomonas after the arrival of an infected case was an impressive contrast to the earlier work. Pseudomonas could be readily isolated from essentially all

objects in direct contact with the patient. Surfaces appear to be the most readily contaminated of all materials tested. Overbed tables, equipment, bedspreads and floors within a six foot radius of the bed yielded Pseudomonas. Hands of personnel were found to be contaminated if tested within 10 minutes after handling the patient. If this time was extended to 1 hour, no Pseudomonas could be detected on the hands. Of interest in this connection was the testing of solutions and medications. The solutions and medications cart was situated approximately 10 feet from the patient's bed during this experimentation. In her usual routine the nurse was observed to move directly from the patient to this table where she would obtain and dispense the various preparations and return to the patient. Yet in no instance was Pseudomonas recovered from these materials during any phase of the study despite the recent findings of Moore et al(19) in regard to hand cream contamination.

The most consistent retrieval and highest counts of Pseudomonas were made from the inhalation therapy equipment. This was probably the most expected result of the overall study. The literature on the communicability of Pseudomonas in hospitals is replete with titles relating to anesthesia and inhalation therapy equipment (6), (12), (16), (17) and (28). Since the patient was suffering from a respiratory Pseudomonas affliction, cultures of this

equipment consistently yielded high counts.

In spite of the intermittent positive pressure and the intervals of stasis, the organisms apparently have no difficulty migrating up the inhalations tubing against the pressure, through the reflux trap and down to the nebulizer kettle. The kettle, acting as a steam supply, moisturizes the output of air. It was sampled after cooling and on one occasion did yield Pseudomonas. The inlet tubing, packed with a copper wool filter material, was uniformly negative.

Judging from the pyocine typing, it appears likely the patient "brought" the organism to the unit. It may have been an endogenous commensal or a smoldering sub-clinical infection when he arrived.

The final sampling period, after the patient expired, showed a sudden shift to the pre-Pseudomonas patient level. A gradual reduction in colonization was not evident. In retrospect, the sampling periods after expiration should have been shortened to perhaps 6 hours, 12 hours and 24 hours. This might have indicated the gradual leveling-off data anticipated which Hurst(9) obtained in his investigation.

The unit tested in this study was a small ward of 12 patients. Considering the entire hospital, with a census of over 300 patients, it is apparent that the Pseudomonas reservoir must be substantial. Hospital

acquired infection caused by the organism will probably persist as an endemic problem which may turn epidemic on occasion. Hopefully, our increasing experience with this infection will lead us to abandon the false sense of security toward Pseudomonas which we have held for so long.

S U M M A R Y

The colonizing habits of Pseudomonas in an intensive care unit of a general hospital was the objective of this study. Sampling was performed during three different periods: (1) a quiescent period when the unit was free of diagnosed Pseudomonas infections, (2) an active period when the unit admitted a respiratory case of Ps. aeruginosa, and (3) a period after the death of the patient when Pseudomonas levels returned to those of the quiescent period.

During each of these periods the unit was sampled with regard to five particular aspects of the environment: surfaces, personnel, water sources, inhalation therapy equipment, and solutions and medications. In the quiescent and decline period, that is before and after the presence of an infected case, Pseudomonas was infrequently isolated. The only areas consistently yielding the organism were the sink drains and the toilet bowls. On the other hand, sampling during the presence of a case yielded these organisms from a diverse number of objects.

In conclusion it can be postulated that Pseudomonas is readily desiccated and only survives for a short time on dry inanimate objects. In a ward situation, a cross infection to susceptible patients would appear likely since the organism does colonize a six foot radius of the patient's

bed.

Inhalation therapy equipment appears to be a unique problem. Pseudomonas colonizes the equipment soon after use by an infected patient and if the apparatus remains at the bedside for several days the levels can become an increasing hazard. Such equipment is often too large or intricate to be sterilized easily after use. At the same time, due to a shortage of equipment in some hospitals, it may not be dismantled and disinfected as often as necessary since it must be available for critical patients whenever needed.

In this investigation it was not unusual to extract a flush of green water from the corrugated tubing of the inhalation therapy equipment and find Pseudomonas colonies too numerous to count. From this investigators' viewpoint this equipment, intimately connected to the respiratory system and harboring the bacteria indicated, is one of the greatest microbiological hazards in an intensive care unit.

L I T E R A T U R E C I T E D

1. Anderson, K. 1959. Pseudomonas pyocyaneus dissemination from an air cooling apparatus. Med. J. Aust. I: 529-533

2. Brown, M. R. 1964. The affect of polysorbate on the growth rate of Pseudomonas aeruginosa. J. Pharm. Pharmacology. 16: Suppl: 51-55.

3. Cross, D. F., A. Benichimol, and E. G. Dimond. 1966. Faucet aerator - source of Pseudomonas infection. New. Eng. J. Med. 274: 1430-1431.

4. Darrell, J. H., and A. H. Wahba. 1964. Pyocine typing of hospital strains of Pseudomonas pyocyanea. J. Clin. Path. 17: 236-242.

5. Drake, C. H. 1966. Evaluation of culture media for the isolation and enumeration of Pseudomonas aeruginosa. Health Lab. Science. 3: 10-19.

6. Frier, J., P. Taylor, and H. M. Gezon. 1967. Pseudomonas aeruginosa epidemic traced to delivery room resuscitators. New Eng. J. Med. 276: 991-996.

7. Frobisher, M. 1950. Fundamentals of bacteriology, p. 572-573. W. B. Saunders Co., Phila.

8. Gilles, R. R., and J. R. W. Govan. 1966. Typing of Pseudomonas pyocyanea by pyocine production. J. Path. Bacterio. 91: 339-345.

9. Hurst, V., and V. L. Sutter, 1966, Survival of Pseudomonas aeruginosa in the hospital environment. J. Infect. Dis. 116: 151-154.

10. Jordan, E. C., and W. Burrows. 1945. Textbook of bacteriology, p. 475-477. W. B. Saunders Co., Phila.

11. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. and Clin. Med. 44: 301-307.

12. Kundsin, R. B., and C. W. Walter. 1962. Asepsis for inhalation therapy. Anesthesiol. 23: No. 4, 507-512.

13. Lee, J. C., and P. J. Fielbow. 1961. Benzalkonium chloride - source of hospital infection with gram-negative bacteria. J. A. M. A. 177: 708-710.

14. Lowbury, E. J., and J. Fox. 1954. The epidemiology of infection with Pseudomonas pyocyaneus in a burns unit. J. Hyg. 52: 403-406.

15. McLoed, J. W. 1958. The hospital urine bottle and bedpan as reservoirs of infection by Pseudomonas aeruginosa. Lancet. I: 394-397.

16. Mertz, J. J., M. L. Scharer, and J. H. McClement, 1967. A hospital outbreak of Klebsiella pneumoniae from inhalation therapy with contaminated aerosol solutions. Amer. Rev. Resp. Dis. 95: 454-460.

17. Moffet, H. L., D. Allan, and T. Williams. 1967. Survival and dissemination of bacteria in nebulizers and incubators. Amer. J. Dis. Child. 14: 13-17.

18. Moffet, H. L., and T. Williams. 1967. Bacteria recovered from distilled water and inhalation therapy equipment. Amer. J. Dis. Child. 114: 7-12.

19. Morse, L. J., and L. E. Schonobeck. 1968. Hand lotions-a potential nosocomial hazard. New Eng. J. Med. 278: 364-369.

20. Morse, L. J., H. L. Williams, F. P. Grenn, E. E. Eldridge, and J. R. Rotta. 1967. Septicemia due to Klebsiella pneumoniae originating from a hand lotion cream dispenser. New Eng. J. Med. 277: 472-473.

21. Osman, M. A. 1965. Pyocine typing of Pseudomonas aeruginosa. J. Clin. Path. 18: 200-202.

22. Park, W. H., and A. W. Williams. 1933. Pathogenic microorganisms, p. 463-465. Lea and Febiger, Phila.

23. Sandusky, W. R. 1961. Pseudomonas infections: sources and cultural data in a general hospital with particular reference to surgical infections. Annals of Surgery. 153: 996-1005.

24. Shaffer, J. G., and M. Coldin. 1962. Hospital epidemiology, p. 8 23-838. In I Davidsohn, and B. B. Wells (ed.), Clinical diagnosis by laboratory methods. W. B. Saunders Co., Phila.

25. [illegible], R. A., K. A. Walker, V. R. Williams, M. T. Parker, E. H. Ashcshov, and J. F. Bullimore. 1966. Faecal carriage of Pseudomonas aeruginosa in hospital patients. Lancet. 2: 1331-1334.

26. Smith, D. T., and N. F. Conant. 1957. Zinsser bacteriology, p. 425-427. Appleton-Century-Crofts, Inc., New York.

27. Stanier, R. Y. 1950. Acetic acid production from ethanol by fluorescent Pseudomonas. J. Bacteriol. 54: 191-194.

28. Sutter, V. L., V. Hurst, M. Grossman, and R. Colonjo. 1966. Source and significance of Pseudomonas aeruginosa in sputum. J. A. M. A. 197: 854-858.

29. Wilson, G. S., and A. A. Miles. 1964. Principles of bacteriology and immunity, p. 636-647. Williams and Wilkins Co., Balt.